Comparative Effects of Bisphenol A, Carbimazole and Thyroxine administration on the Thyroid Gland, Serum Selenium and Iodine Concentration of Wistar Rats

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Abstract

The effects of Bisphenol A (BPA), carbimazole and thyroxine on thyroid gland were investigated in 40 Wistar rats assigned as untreated controls, BPA (20 µg/kg/day), carbimazole (5 mg/day), and thyroxine (25 µg/day) orally by gavage. Each group contained 10 rats, 5 males and 5 females. BPA and carbimazole caused hypothyroidism within 30 days. The concentrations of T_3 and T_4 were lower (P<0.05-0.001) in the groups received BPA and carbimazole, respectively, in both males and females. Significant increases (P<0.05-0.01) of the two hormones were observed in rats given thyroxine at 25µg/day than the control rats. TSH concentration decreased (P<0.01) in the rats received thyroxine, while significant increases (P<0.05-0.01) were observed in the rats received BPA and carbimazole, respectively, than the controls. Thyroxine was found to induce hyperthyroidism as evidenced by elevation of T_4 and T_3 with a significant decrease of TSH. The concentrations of selenium and iodine were lower (P<0.05-0.01) in the test groups of both sexes than the control rats. The group received thyroxine, scored the lowest selenium concentration of all the other test groups and significantly lower (P<0.01) than the control, while those received carbimazole showed lower (P<0.05-0.01) iodine levels. No change was observed in the iodine level of the female rats received thyroxine. Alteration in serum thyroid hormones, selenium and iodine concentrations were corelated with changes in thyroid histological structure. Only on rat of BPA received group died one day before the end of the experimental period.

Keywords: Bisphenol A, Thyroid hormones, Thyroxin, Carbimazole, Selenium, Iodine.

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1. Introduction

Bisphenol A (BPA), an estrogenic endocrine-disrupting chemical (EDC) is used to manufacture polycarbonate plastic and epoxy resins which are used as coatings in the food-packing industry. Polymerization of epoxy resin reactions were known not be fully complete, and that a considerable proportion of unreacted epoxy compounds can be recovered from food packed in containers lined with these plastics [1], [2]. BPA can be released into the environment during the manufacturing process and by leaching from the final products [3], [4], [5]. The migration of cured resin components into foods has also been reported. Unreacted epoxy compounds are thought to be toxic due to their alkylating properties [6]. In the environment it can be detected in air, soil and aquatic ecosystem [7], and for all these reasons it is not surprising that it has been identified also in human tissues and fluids. BPA has well characterized estrogenic and other endocrine disrupting activities that are mediated via multiple molecular mechanisms, mainly nuclear receptor signaling pathways [8]. The antagonism with thyroid receptors (TRs), which affects TR-mediated transcriptional activity, the immediate action of BPs on the expression of genes at the thyroid and also the pituitary level, the competitive binding with thyroid transport proteins, and the toxicity induction in several cell lines are likely the major mechanisms resulting in thyroid dysfunction. In humans, results are more contradictory, though some evidence suggests the potential of BPs in increasing the risk of thyroid nodules [9]. There is evidence for an anti-thyroid hormone effect of BPA leading to the reduction of the thyroid hormone (TH) mediated gene expression by enhancing the TH receptor (TR) interaction with a transcriptional co-repressor [10]. Li *et a*l. [11] reported the evidence that BPA as environmental risk factor can facilitate the progression Papillary Thyroid Carcinoma (PTC) harboring the $BRAF^{V600E}$ mutation. THs regulate a variety of biological processes associated with metabolism, energy provision, development, somatic growth, and reproduction in vertebrates and, thus, effects of EDCs on the thyroid system may pose a hazard to human and wildlife health [12], [13]. The objective of this study was to evaluate the effects of low dose of BPA on thyroid structure and hormones, serum selenium and iodine concentrations as compared to exogenous hypothyroid (carbimazole) and hyperthyroid (thyroxine) induction.

2.Materials and methods

The present study was carried out in the Department of Biochemistry and Molecular Biology, Faculty of Science and Technology, El Neelain, University, Sudan, after getting approval from Scientific Research Ethical Committee. Forty Wistar rats were obtained from the Faculty of Pharmacy University of Khartoum, reared within the premises of the animal house under 12 hours photoperiod with standard feed and drinking water provided *ad libitum* before the commencement of experimental feeding. Room temperature was maintained at 25±2 0C at adequate house ventilation. Then the animals were randomly allotted into four groups 1, 2, 3, and 4 each of ten rats (5 males and 5 females). Group 1 was designated as the control group. Extra Pure (97%) Bisphenol A powder (Sangon, China) was thoroughly dissolved in distilled water and rats of group 2 received doses at 25µg /kg body weight/day, group 3 received carbimazole at (5 mg/day) and group 4 was given thyroxin at (25 µg/day). The treatment groups received their test materials and the control given normal saline by oral gavage for a period of 4 weeks.

2.1 Data collection

2.1.1 Serum analysis

After the end of the experimental period, rats of the control and treatment groups were anaesthetized with diethyl ether and humanely slaughtered. Blood was collected at slaughter in clean sterile vials and sera were separated thereafter to be analyzed for the thyroid hormones, Thyroxin (T_4), Triiodothyronine (T_3) and Thyroid Stimulating Hormone (TSH) according to Aviva Systems Biology [14]. Selenium and iodine concentration were analyzed using the Inductively Coupled Plasma-Mass Spectrometry (ICP-MS)-mayo clinic laboratory [15].

2.1.2 Histopathological methods

Necropsy was conducted to identify gross lesions and specimens of thyroid gland were immediately being collected immediately after slaughter of rats, fixed in 10% neutral buffered formalin and embedded in paraffin wax, sectioned at 5µm and stained with Hematoxylin and Eosin (H & E) [16].

2.2 Statistical analysis

Mean values of Thyroxin (T_4), Triiodothyronine (T_3), Thyroid Stimulating Hormone (TSH), serum selenium and iodine concentration, were compared using student's t-test [17].

3. Result

3.1 Clinical observations

The control group 1 remained clinically normal throughout the experimental period. On the fourth day of the experiment, rats of group 2 showed aggressiveness behavior and dosing resistance in addition to inappetence. On the last day of experimental period one rat from groups 2 died.

3.2 Serum thyroid hormones concentration

Changes in the concentration of T_3, T_4 and TSH in male and female rats are presented in Tables 1 & 2, respectively. The trend of increment and decrement in the thyroid hormone levels is similar in both male and female Wistar rat groups when BPA, carbimazole and thyroxine were administered. By the end of week 4 experimental period, the concentration of T_3 and T_4 were lower (P<0.05-0.001) in groups 2 received BPA at 25µg /kg body weight/day and carbimazole at 5 mg/day, respectively, in both males and females. Significant increases (P<0.05-0.01) of the two hormones were observed in group 4 (males and females) given thyroxine at 25µg/day than the control rats of group 1. TSH concentration decreased (P<0.01) in group 4 received thyroxine at 25µg/day while significant increases (P<0.05-0.01) were observed in group 2 and 3 received BPA at 20µg /kg body weight/day and carbimazole at 5mg/day, respectively than the control group 1.

Table 1: Changes in serum Thyroxine (T_4), Triiodothyronine (T_3) and Thyroid Stimulating Hormones (TSH) in male (m) Wistar rats

Group No.	Dose	T_3 ng/dL	T_4 ng/dL	TSH µIU/mL
1m	**Control**	104.21±0.12	111.78±0.21	0.041±0.14
2m	**BPA (25µg/kg/day)**	84.99±0.11**	93.60±0.52**	0.049±0.13*
3m	**Carbimazole (5mg/day)**	85.12±0.09**	84.87±0.15***	0.053±0.60**

4m	**Thyroxine (25µg/day)**	123.09±0.67**	115.04±0.13*	0.018±0.98**

NS = not significant, *Denotes mean values significant at (P<0.05), **Significant= (P<0.01), *** Significant= (P<0.001).

Table 2: Changes in serum Thyroxine (T_4), Triiodothyronine (T_3) and Thyroid Stimulating Hormones (TSH) in female (f) Wistar rats

Group No.	Dose	T_3 ng/dL	T_4 ng/dL	TSH µIU/mL
1f	**Control**	108.12±0.89	110.09±0.17	0.031±0.71
2f	**BPA (25µg/kg/day)**	83.04±0.12**	96.13±0.43**	0.047±0.42*
3f	**Carbimazole (5mg/day)**	86.76±0.89**	93.41±0.29**	0.046±0.54*
4f	**Thyroxine (25µg/day)**	125.32±0.21**	118.43±0.56*	0.023±0.07**

NS = not significant, *Denotes mean values significant at (P<0.05), **Significant= (P<0.01).

3.3 Concentrations of selenium and iodine

Changes in the concentrations of serum selenium and iodine of the control, males and females test groups are presented in Table 3 &4. Except for group 4 female rats (Table 4), the concentrations of selenium and iodine were lower (P<0.05-0.01) in the test groups of both sexes than the control rats. Group 4 of both sexes scored the lowest selenium concentration of all the other test groups and significantly lower (P<0.01) than the control, while group 3 showed lower (P<0.05-0.01) iodine levels. No change was observed in the iodine level of group 4 female rats.

Table (3): Concentration of serum selenium and iodine in BPA, carbimazole and Thyroxine in male Wistar rats

Group	Administered dose	Selenium µg/ml	Iodine µg/ml
1 Control	Nil	0.41±0.12	0.17±0.89
2	Bisphenol A 25 µg/kg/day	0.37±0.17*	0.15±0.11*

3	Carbimazole 5 mg/day	0.36±0.43*	0.11±0.02**
4	Thyroxine 25 µg/day	0.25±0.24**	0.12±0.76*

NS = not significant, *Denotes mean values significant at (P<0.05), ** Significant= (P<0.01).

Table (4): Concentration of serum selenium and iodine in BPA, carbimazole and Thyroxine in female Wistar rats

Group	**Administered dose**	**Selenium µg/ml**	**Iodine µg/ml**
1 Control	Nil	0.44±0.43	0.18±0.78
2	Bisphenol A 25 µg/kg/day	0.32±0.39**	0.13±0.96*
3	Carbimazole 5 mg/day	0.39±0.89*	0.12±0.54*
4	Thyroxine 25 µg/day	0.28±0.01**	0.19±0.76^{NS}

NS = not significant, *Denotes mean values significant at (P<0.05), ** Significant= (P<0.01).

3.4 Thyroid histopathology

In the control group 1, the thyroid gland remained normal throughout the experimental period (Fig. 1 & 2). In the thyroid gland of female rats of group 2, the majority of the thyroid follicles varied greatly in size and colloid contents with lymphocytic infiltration. Damaged follicles were also seen (Fig3). In Male rats of group 2 the thyroid follicles also varied in size and colloid content, follicles and follicular epithelium were damaged with aggregates of lymphocytes (Fig. 4). The thyroid gland of carbimazole- treated rats of both sexes were affected. In male rats of group 3 some of the thyroid follicles become dilated with varying amount and densely stained colloid and many other appeared smaller in size, aggregates of lymphocytes were also observed (Fig. 5). In female rats of group 3, some of the thyroid follicles were enlarged with slightly stained colloid and other follicle appeared smaller in size with marked lymphocytic infiltration in the interstitium (Fig. 6). The administration of thyroxine also affected the structure of the thyroid gland of the treated rats of group 4. Follicular damage and diffuse hyperplasia were observed in the thyroid glands of female rats of group 4 received thyroxine at 25 µg/kg/day as evident in (Fig. 7). Follicular damage and accumulation of lymphocytes and fibroblasts were observed also in male rats given the same above dose of thyroxine (Fig. 8)

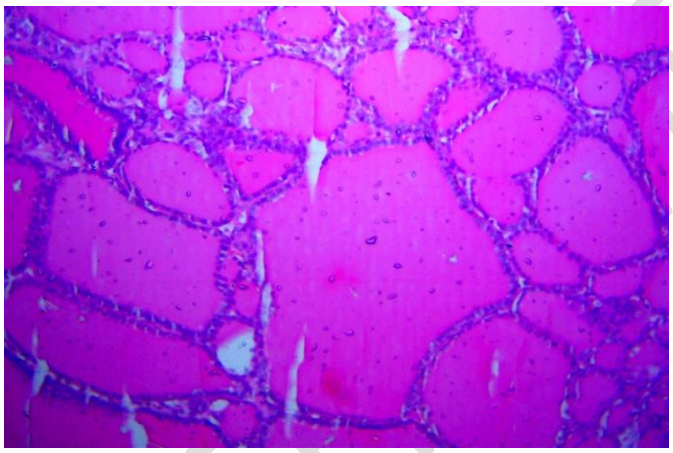

Fig.1: Thyroid gland of male control rats of group 1. H & E X120

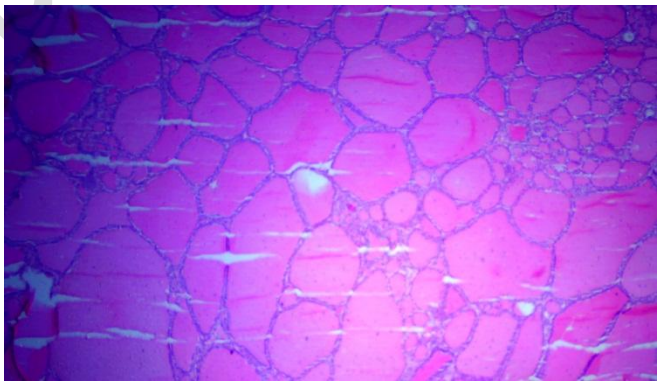

Fig.2: Thyroid gland of female control rats of group 1. H & E X120

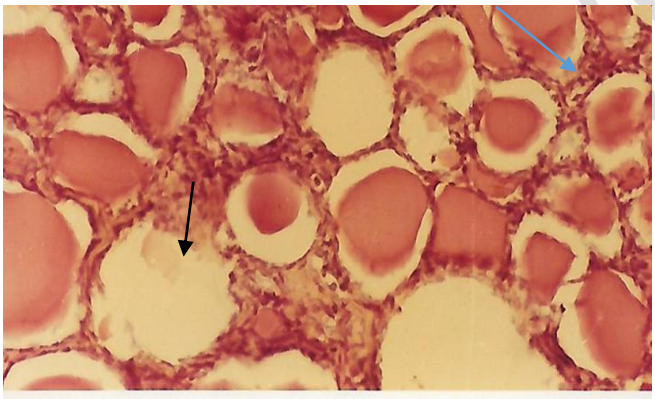

Fig 3: Thyroid gland of female rat group 2 received BPA at 25µg /kg body weight/day showing follicular damage (black arrow) with little lymphoid cell accumulation (blue arrow). H & E ×120

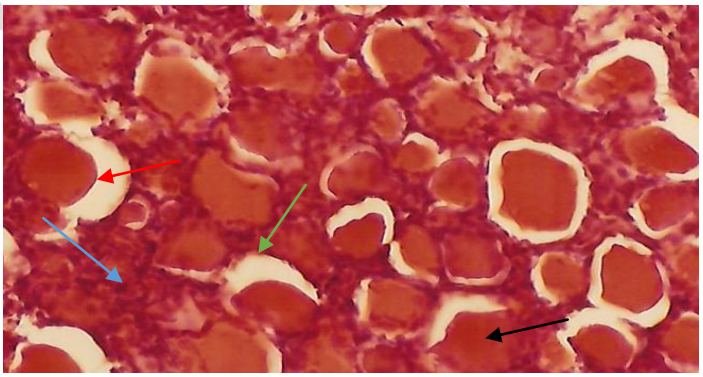

Fig 4: Thyroid gland of male rats of group 2 given BPA at 25µg /kg body weight/day showing colloid shrinking (red arrow), damaged follicles (black arrow) and follicular epithelium (green arrow) with aggregates of lymphocytes (blue arrow). H & E X 120

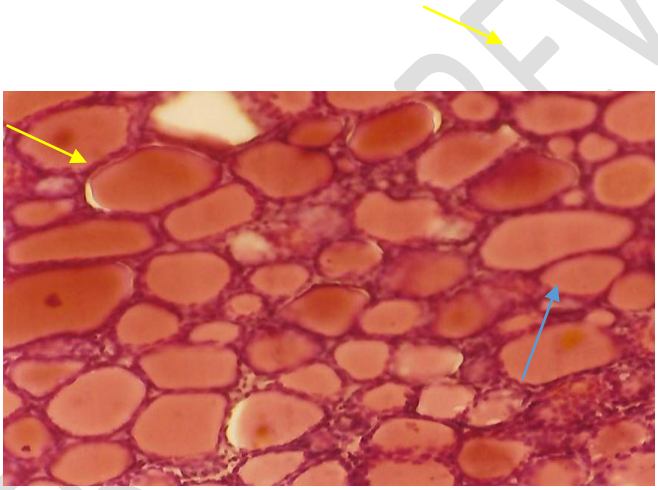

Fig. 5: Small and elongated follicles with densely stained colloid (yellow arrow) and aggregates of lymphocytes (blue arrow) in the thyroid gland of male rats of group 3 given carbimazole at 5 mg/day. H & Ex120.

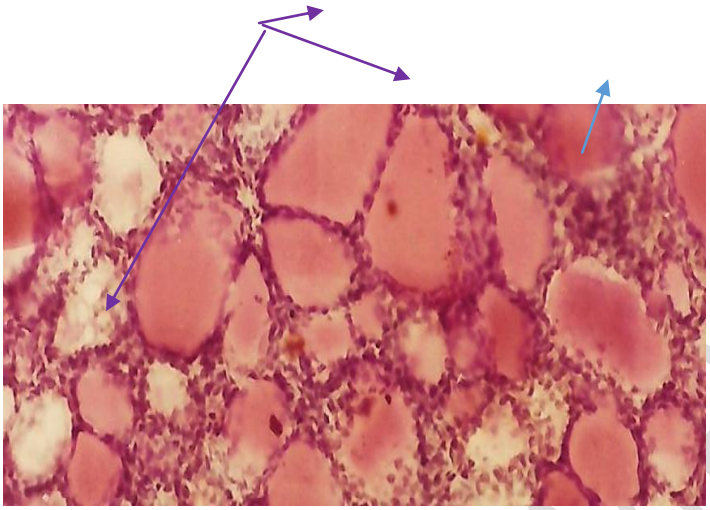

Fig. 6: Lightly stained large follicles (violet arrow) and small sized ones with marked interstitial lymphocytic infiltration (blue arrow) in female rats of group 3 received carbimazole at 5 mg/day. H & Ex120.

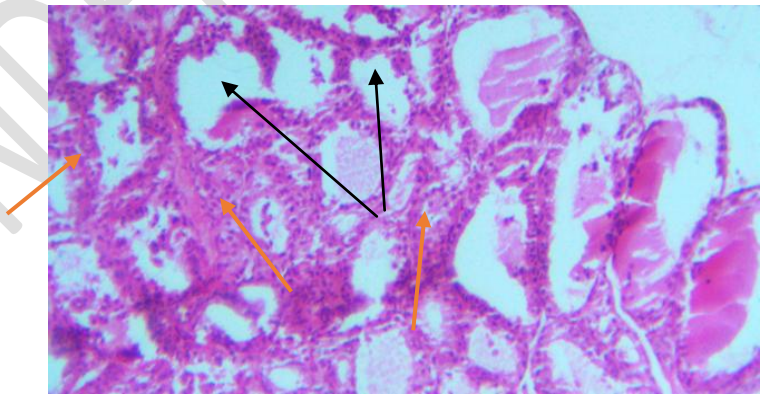

Fig. 7: Diffuse thyroid hyperplasia **(**orange arrow**)** and damaged follicles (black arrow) in female rats of group 4 given thyroxine at 25 µg/day. H & Ex120

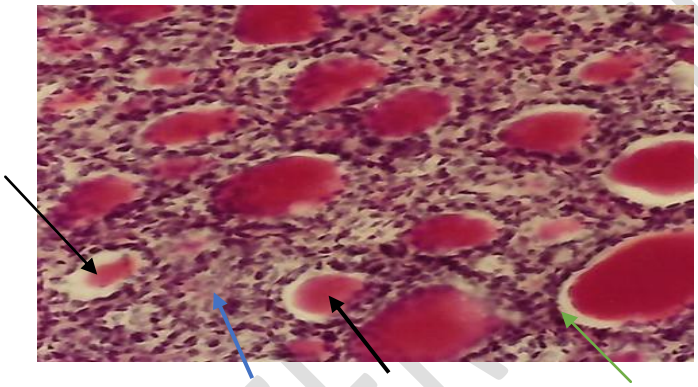

Fig. 8: Damaged thyroid follicles (black arrow) with lymphocytic accumulation and deposition of some fibroblast (blue arrow) and degeneration of follicular epithelium (green arrow) in male rats of group 4 given thyroxine at 25 µg/day. H & Ex120.

4. Discussion:

Endocrine Disrupter Chemicals (EDCs), which modify natural endocrine function, have emerged as a major public health issue due to their potentially disruptive effects on physiological processes, particularly through direct interaction with steroid hormone receptors [18]. Some of these EDCs were reported to be responsible for disruption of thyroid system function [19] [20].

Thyroid hormones (thyroxine T_4 and triiodothyronine T_3) are essential for normal behavioral, intellectual, and neurological development. Congenital hypothyroidism, if left untreated, causes irreversible mental retardation. Even mild maternal thyroid deficiency during pregnancy could cause retarded neurological development of the child [21]. The results of the present study

indicated the anti-thyroid effect of low dose of BPA (25 µg/kg/day) for Wistar rats of both sexes where thyroid gland microscopic and functional alteration were observed. That was evidenced by the decreased concentration of T_4 and T_3 with a significant increased concentration of TSH in serum of BPA- treated animals in addition to degeneration of the thyroid follicles with lymphocytic infiltration. However, neoplasia was not detected in this group.

In thyroid hormone synthesis, iodine enters thyrocytes via the sodium iodide symporter (NIS), is oxidized by Thyroid Peroxidase (TPO), and is incorporated into tyrosyl residues of thyroglobulin (*Tg*) [22]. BPA exposure has been found to change the expression of genes involved in these processes, such as NIS *Slc5a5* (solute carrier family 5 member 5), *Tpo*, and *Tg*. *In vitro* (FRTL5 cells) and *in vivo* (zebrafish) models were used by Genticore *et al* [23] to examine the effects of BPA in regulating the expression of the genes involved in thyroid hormone synthesis and of their transcriptional regulators at BPA as low as 25 µg/kg/day. In both systems, altered expression in the genes involved in thyroid hormones synthesis was detected. Also, the direct effect on thyroid follicular cells, which are affected by very low amount of BPA, was observed as well. Thus, their results potentially, represent an ideal *in vitro* system to develop assays to detect BPA and other pollutants with thyroid disrupting activity at level far below the ones considered to be environmental relevant. Moreover, this may provide new insight into the mode of BPA-induced deregulation of physiological processes as well as on the extensively debated molecular pathways underlying its biological activities. The authors also reported BPA to inhibit TR-mediated transcription by acting as an antagonist and suppressed transcriptional activity that is stimulated by thyroid hormone (T_3) in a dose-dependent manner.

Low concentrations of BPA were found to suppress the (Thyroid Receptor) TR transcription by disrupting physiologic concentrations of T_3/T_4-mediated β3 integrin/c-Src/MAPK/TR-β1 pathways, followed by recruiting N-CoR/SMRT to TR-β1, providing a novel insight regarding the TH disruption effects of low concentration BPA [24]. A study conducted by da Silva *et al*. [25] revealed that the exposition of thyrocytes to the endocrine disruptor bisphenol A can increase Reactive Oxygen Species (ROS) production, both *in vivo* and *in vitro*. Moreover, BPA decreased thyroid iodide uptake and thyroperoxidase activity, two essential steps for Thyroid hormone (TH) synthesis. The authors related this effect to an increased oxidative stress since N-acetylcysteine (NAC) could prevent the reduction of thyroperoxidase (*Tpo*) and sodium-iodide symporter (*Nis*) mRNA levels induced by BPA in PCCL3 (rat thyroid cell line). The

enhancement of ROS production by thyrocytes related to the exposition to BPA could lead to oxidative damage of the gland, thus predisposing individuals exposed to BPA to thyroid diseases.

Many human and animal BPA administration experiments have been conducted. In rodents, most of the studies have been conducted in pregnant females, and have assessed the variations of TH levels in mothers and offspring following prenatal and/or lactational exposures. It was reported by Fernandez *et al.* [26] that neonatal exposure to BPA alters the hypothalamic-pituitary-thyroid axis in adult female rats and females exposed to the medium dose of BPA at 50μg 50 μl^{-1} oil exhibit the highest TSH levels, whereas slight increase was observed in high 500μg 50 μl^{-1} and low doses (5μg 50 μl^{-1}). [27] and [28] observed a significant increase of serum T4 levels in pups of both sexes and in female adults, respectively, without any apparent interference on TSH release. A decrease in T4 levels was found in male and female adult rodents [29], [30] and in rat pups of both sexes [31] or with a sex-specific effect [32]. In 2019, da Silva *et al.* [33] and others confirmed the capability of BPA to inhibit both type 1 and type 2 deiodinases, enzymes responsible for the conversion of the pro-hormone T_4 into the biologically active thyroid hormone T_3. *In vivo* bisphenol A administration at 40 mg/kg body weight significantly reduced hepatic type 1 deiodinase activity, increased serum T_4 levels, while T_3 remained unchanged. T_3/T_4 ratio was decreased in rats treated with bisphenol A, reinforcing the idea that peripheral metabolism of thyroid hormone was affected by bisphenol A exposure. Therefore, their results suggest that bisphenol A can affect the metabolism of thyroid hormone thus disrupting thyroid signaling. The inconsistent results of these experiments might be attributed to different doses, routes of exposure to BPA in addition to environmental condition which can induce stress to experimental animals.

Experimentally, suppression of hormone production has been the base for studying thyroid dysfunctions and changes in the metabolism and body development [34] [35], or thyroxin administration in high doses inducing hyperthyroidism status [36] [37]. For unknown reasons resistance to induction of hypothyroidism in mice was described in the literature [38]. In 2007 Ferreira *et al.*[37] established a protocol to induce hyperthyroidism and hypothyroidism in adult female mice, in a simple and practical way, in order to obtain an experimental model.

The induction of hypothyroidism (carbimazole) and hyperthyroidism (Thyroxine) in treated rats was confirmed by the functional and histopathological alteration of the thyroid gland at the end

of the experimental period. Carbimazole is an antithyroid drug similar to methimazole i.e. a prodrug converted to methimazole after administration. The mode of action of carbimazole is to act as an inhibitor for thyroid peroxidase (TPO) and decreases incorporation of iodide into tyrosine molecules. It also inhibits coupling of mono-iodinated and di-iodinated residues to form T_4 and T_3. Carbimazole has been the drug of choice in some hyperthyroid patients because it may have fewer side effects, such as less frequent gastrointestinal tract problems [39]. Thyroidal effects produced by carbimazole, at the end of the experimental period, were similar to those produced by BPA *i.e.* hypothyroidism.

L-thyroxine, used for induction of hyperthyroidism in this study, is a synthetic form of the thyroid hormone thyroxine and is often used for the treatment of hypothyroidism and thyroid hormone deficiency. It has the ability to lower the thyroid-stimulating hormone (TSH), a hormone that is considered goiter-inducing [40].

The relation between selenium and iodine has been well studied [41] [42]. The authors found that selenium is needed for hepatic conversion of thyroxine (T_4) to 3,3,5-triiodothyronine(T_3) and that type 1& 2 iodothyronine deiodinases, identified as a selenocysteine containing enzymes, catalyze deiodination of (T_4) to biologically active thyroid hormone (T_3) and thus play an important role in thyroid hormone metabolism.

5. Conclusion

The results of the present study indicated the hypothyroid effect of low dose of BPA for Wistar rats of both sexes (25 µg/kg/day) where the thyroid gland microscopic and functional alteration were observed. That was evidenced by the decreased concentration of thyroid hormone and increase of thyroid stimulating hormone.

Ethical approval

Animal ethic Committee approval has been collected and preserved by the author.

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